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REPORT DOCUMENTATION PAGE

AD-A201 735

1988

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S): ARO 22203.9-LS
6a. NAME OF PERFORMING ORGANIZATION: Boston University School of Medicine	6b. OFFICE SYMBOL (If applicable)
6c. ADDRESS (City, State, and ZIP Code): 80 East Concord Street, Boston, MA 02118	7a. NAME OF MONITORING ORGANIZATION: U. S. Army Research Office
	7b. ADDRESS (City, State, and ZIP Code): P. O. Box 12211, Research Triangle Park, NC 27709-2211
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U. S. Army Research Office	8b. OFFICE SYMBOL (If applicable)
8c. ADDRESS (City, State, and ZIP Code): P. O. Box 12211, Research Triangle Park, NC 27709-2211	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract/Grant #DAAG29-85-K-0071
	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification): Molecular Probe Analysis of Mammalian Brain Acetylcholinesterase

12. PERSONAL AUTHOR(S): Judith K. Marquis, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 3-1-85 TO 10/31/88	88/09/27	13

16. SUPPLEMENTARY NOTATION: The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

17. COSATI CODES: FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Acetylcholinesterase, Organophosphates, Neurotoxicity, Chemical modification, Tetrahydroaminoacridine

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the project period covered by this contract, our work emphasized studies of the kinetic properties of purified bovine brain acetylcholinesterase (AChE) and experiments to chemically modify the reactivity of this enzyme. Our goal has been to define the properties of peripheral or extra-catalytic sites on AChE in the hope that chemical modification of organophosphate (OP) inhibition can be achieved through binding at the peripheral sites. It is expected that susceptibility to OP inhibition can be reduced while maintaining the enzyme's catalytic activity.

Among the peripheral site ligands studied, tetrahydroaminoacridine and lucanthone provided the most promising evidence of pharmacological modification. In addition, a number of non-pharmacological compounds, i.e., nonselective and quite neurotoxic compounds, such as the water-soluble carbodiimides and phenylglyoxal (an arginine group ligand), also reduced AChE susceptibility to certain phosphorylating inhibitors.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Judith K. Marquis, Ph.D.	22b. TELEPHONE (Include Area Code): (617) 638-5142 / 22c. OFFICE SYMBOL

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SECURITY CLASSIFICATION OF THIS PAGE: UNCLASSIFIED

ARO 22203.9-LS

TITLE

Molecular Probe Analysis of Mammalian Brain Acetylcholinesterase

TYPE OF REPORT (TECHNICAL, FINAL, ETC.)

Final

AUTHOR (S)

Judith K. Marquis, Ph.D.

DATE

September 27, 1988

U. S. ARMY RESEARCH OFFICE

CONTRACT/ GRANT NUMBER

DAAG29-85-K-0071

INSTITUTION

Boston University School of Medicine

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22203-LS

FINAL TECHNICAL REPORT

1. ARO PROPOSAL NUMBER: 22203-LS

2. PERIOD COVERED BY REPORT: 1 March, 1985 - 30 June, 1988

3. TITLE OF PROPOSAL: Molecular Probe Analysis of Mammalian Brain Acetylcholinesterase.

4. CONTRACT OR GRANT NUMBER: DAAG29-85-K-0071

5. NAME OF INSTITUTION: Boston University School of Medicine, Boston, MA 02118.

6. AUTHOR OF REPORT: Judith K. Marquis, Ph.D.

7. List of Manuscripts Submitted or Published Under ARO Sponsorship during this Reporting Period, Including Journal References: See Appendix A.

List of Abstracts of Papers Presented at Scientific Meetings Under ARO Sponsorship during this Reporting Period, Including Journal References: See Appendix B.

List of Seminars and Lectures Presented Under ARO Sponsorship during this Reporting Period, Including Titles, Dates, Locations: See Appendix C.

8. Scientific Personnel Supported by this Project and Degrees Awarded During this Reporting Period:

Judith K. Marquis, Principal Investigator
Thomas Biagioni, Senior Research Technician
Robert MacCallum, Research Technician
Gordon Siek, Graduate Student
Charles Cyr, Graduate Student, D.O. Awarded
Eric Fishman, Graduate Student, M.S. Awarded
Anthony Korosi, Student.

PROGRESS REPORT: SUMMARY OF RESEARCH FINDINGS

Introduction.

This grant was originally funded for a 3-year work period, the time requested as adequate to carry out the studies described in the Statement of Work of the project proposal. After the first 2 years of funding, however, ARO reduced the third year of funding by 80%. Effectively, then, only a very low level of activity could be maintained during this final (third) year.

Despite this cut in funding, I believe progress in this area of research was significant. In addition, the project fostered a very useful interaction between our neurotoxicology laboratory here at Boston University School of Medicine and several U.S. Army research laboratories, including those at the Natick, Mass., facility, the Institute for Chemical Defense at Aberdeen Proving Ground, and Walter Reed Army Hospital, Washington, DC.

Under the sponsorship of the U.S. Army Research Office, progress was most notable in each of the following areas of neuroscience and, more specifically, neurotoxicology:

A. Studies of the kinetic properties of purified bovine brain acetylcholinesterase (AChE):

Techniques for the following kinetic determinations were established in the laboratory: carbamylation and decarbamylation (primarily with neostigmine); phosphinylation and dephosphinylation (with compounds provided by Dr. Lieske, APG). The data are summarized in Tables I-III as well as in two abstracts of papers presented describing this work; a manuscript is being prepared for publication. In this part of our studies, we evaluated the kinetics of inhibition of control and modified enzyme by organophosphinate compounds and reactivation of the inhibited enzyme, including both spontaneous reactivation and oxime-induced reactivation.

Studies of the aging of diisopropylfluorophosphate (DFP)-inhibited purified bovine caudate AChE: During the period of this project, we established routine assay procedures to measure the rate constant of aging (dealkylation) of phosphorylated enzyme. We used this technique to determine whether pharmacologically relevant chemical modifying reagents can effectively reduce the rate of aging and facilitate oxime reactivation.

Kinetic studies of peripheral site ligands and purified human serum cholinesterase were also conducted during the first year of this project period. These were presented at the 1986 Society of Toxicology meetings in New Orleans and later published as a full length paper in the journal Neurotoxicology. In order to focus on

the brain AChE chemical modification studies, this serum enzyme work was suspended.

B. Separation and identification of the molecular forms of AChE:

Working with bovine caudate nucleus as a source of AChE, we routinely separate the two major molecular forms, G1 and G4, globular monomers and tetramers that predominate in mammalian brain. We have obtained homogeneous fractions of G4 (10s) for kinetic studies, but we are still exploring methods for an efficient separation of a homogeneous fraction of G1 (4s) enzyme.

C. Inhibition of the isolated molecular forms of AChE by carbamates and organophosphates:

We examined the interaction of the carbamates neostigmine and physostigmine and the organophosphates dichlorvos, paraoxon and DFP with AChE, measuring the Ki for each compound, and, where measurable, the rate of reactivation of inhibited enzyme.

D. Chemical modification of peripheral anionic sites or "extra-catalytic" sites on human serum ChE and bovine brain AChE.

This work was a continuation of our earlier studies supported by our previous U.S. ARO research contract (Marquis, Neurotoxicol. 6:261-279, 1985). We expanded that study by focussing on the noncompetitive inhibitor lucanthone and measuring the effects of this very potent peripheral site ligand on the kinetic constants for phosphorylation and carbamylation. While we were able to demonstrate that lucanthone significantly alters enzyme affinity for organophosphates, the nature of that modification is yet to be defined.

Additional studies of site-selective ligands for peripheral anionic sites on AChE: Encouraged by clinical reports that tetrahydroaminoacridine (THA) may alter central cholinergic function in humans, we analyzed the binding sites for this therapeutic agent and found that THA is selectively bound to a subset of the so-called "gamma" anionic sites at a locus distinct from the binding site for aluminum or gallamine. We began studies to analyze the interactions between the THA-binding site and catalytic site reactivity with both organophosphates and organophosphinates. The results were presented at the 3rd ARO Neurosciences Program Review in Cashiers, NC (abstract enclosed), and a full-length manuscript is currently in preparation.

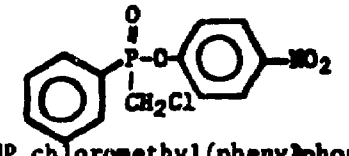
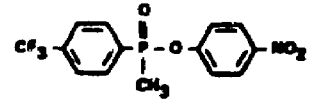
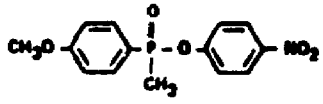
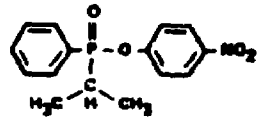
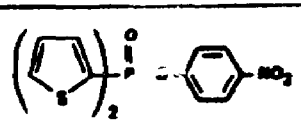
Studies of aluminum binding to peripheral anionic sites on mammalian brain AChE. In the earlier stages of this project, we demonstrated that Al exhibits very high affinity for a specific subset of peripheral anionic sites on AChE. We considered the possibility that AChE represents a biochemical target for Al toxicity, and studied the kinetics of AChE inhibition by Al. Two

reviews of this topic were prepared for publication during this project period. Copies are enclosed. We have continued to pursue our interest in aluminum neurotoxicity with special attention to the notion that environmental toxicants such as Al, that bind to peripheral anionic sites on AChE, may alter the susceptibility of AChE to catalytic site inhibition by organophosphates.

E. Toxicologic Impact of Organophosphate Exposure:

As an extension of our work on AChE inhibitors, including organophosphate and carbamate compounds, we have been working with the U.S. EPA to develop a further understanding of the toxicologic impact of OP exposure as estimated by measuring levels of ChE activity in plasma, erythrocyte, and brain of both laboratory animals and humans. We have addressed the difficulties of species extrapolation, methodology for enzyme monitoring, etc.

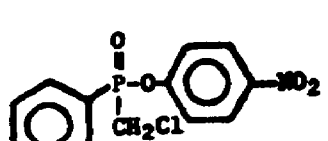
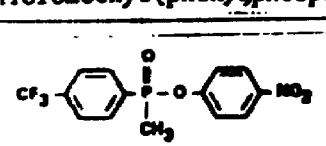
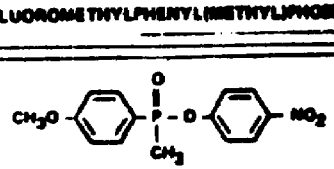
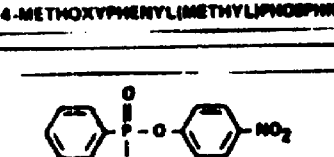
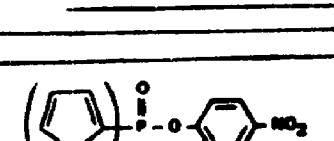
TABLE I. INHIBITION CONSTANTS (K_I) FOR ORGANOPHOSPHINATES AND PURIFIED ACETYLCHOLINESTERASE FROM BOVINE CAUDATE NUCLEUS

ORGANOPHOSPHINATE	CONTROL K_I*	EDAC-TREATED AChE	CMCT-TREATED AChE
 4NP chloromethyl(phenyl)phosphinate	9.9 ± 1.48	11.73 ± 0.45	11.4 ± 0.68
 4NP TRIFLUOROMETHYLPHENYL(METHYL)PHOSPHINATE	8.7 ± 0.25	6.9 ± 0.35 †	11.2 ± 1.5
 4NP 4-METHOXYPHENYL(METHYL)PHOSPHINATE	9.5 ± 0.98	22.8 ± 5.05 †	16.7 ± 1.58 †
 4NP ISOPROPYLPHENYLPHOSPHINATE	2.04 ± 0.03	2.4 ± 0.08 †	2.36 ± 0.23 †
 4NP BIS(2-THIENYL)PHOSPHINATE	2.0 ± 0.13	4.3 ± 0.35 †	3.32 ± 0.2 †

*Units = Moles^{-1} x 10^5 sec^{-1} Values are expressed as mean ± std. dev., $n = 4$:

† $p \leq .05$

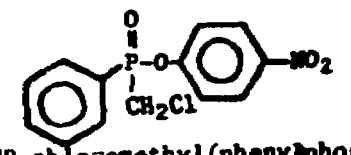
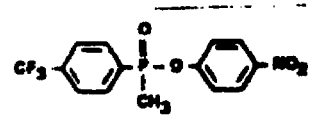
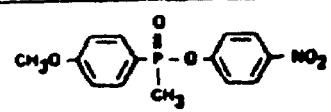
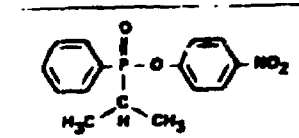
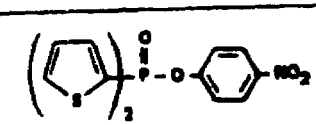
TABLE II. SPONTANEOUS REACTIVATION (%-uninhibited activ) 2 & 6 HOURS FOLLOWING COMPLETE INHIBITION OF PURIFIED BOVINE ACETYLCHOLINESTERASE BY ORGANOPHOSPHINATES.

	CONTROL		EDAC-TREATED		CMCT-TREATED	
	2 HRS	6HRS	2 HRS	6 HRS	2 HRS	6 HRS
 4NP chloromethyl(phenyl)phosphinate	10.1 ± 7.16	42.4 ± 9.5	62.3 ± 41.4	100% †	0	88.4 ± 2.
 4NP TRIFLUOROMETHYLPHENYLMETHYLPHOSPHINATE	12.2 ± 3.25	28.5 ± 15.3	50.7 ± 42.4	93.2 ± 2.6†	65.5 ± 11.5†	100% †
 4NP 4-METHOXYPHENYLMETHYLPHOSPHINATE	12.7 ± 4.9	31.6 ± 14.8	12.5 ± 7.2	20.9 ± 16.8	4.5 ± 0.1†	24.7 ± 0.
 4NP ISOPROPYLPHENYLPHOSPHINATE	5.85 ± 1.91	10.6 ± 3.9	55.9 ± 8.95†	100% †	12.9 ± 1.1	36.4 ± 27
 4NP BIS(2-THIENYL)PHOSPHINATE	17.2 ± 2.4	23.9 ± 14.55	0	78.8 ± 0.2†	0	19.3 ± 0.

† $p < .05$

Values are expressed as mean ± std. dev., n = 3 or 4.

TABLE III. OXIME-INDUCED REACTIVATION (MAXIMUM ACHIEVED, EXPRESSED AS %-CONTROL OR % OF UNINHIBITED ENZYME ACTIVITY) FOR PURIFIED BOVINE CAUDATE ACETYLCHOLINESTERASE FOLLOWING COMPLETE INHIBITION BY ORGANOPHOSHINATES. TIME TO MAXIMUM REACTIVATION IS INDICATED IN PARENTHESES. (EXPERIMENTS WERE TERMINATED AT 90 MINS.)

ORGANOPHOSPHINATE	CONTROL		EDAC-TREATED		CMCT-TREATED	
	2-PAM	OBIDOX	2-PAM	OBIDOX	2-PAM	OBIDOX
 4NP chloromethyl(phenyl)phosphinate	62.7 ± 20.2 (90 mins)	91.1 ± 6.2 (40 mins)	100% † (90 mins)	100% (30 mins)	100% † (90 mins)	87.7 ± 7.3 (30 mins)
 4NP TRIFLUOROMETHYLPHENYL(METHYL)PHOSPHINATE	34.2 ± 13.1 (90 mins)	100% (30 mins)	100% † (90 mins)	100% (50 mins)	52.2 ± 5.0 † (30 mins)	100% (30 mins)
 4NP 4-METHOXYPHENYL(METHYL)PHOSPHINATE	21.4 ± 7.7 (90 mins)	100% (90 mins)	22.3 ± 2.4 (90 mins)	100% (30 mins)	33.5 ± 3.9 † (90 mins)	45.7 ± 36.((90 mins)
 4NP ISOPROPYLPHENYLPHOSPHINATE	41.6 ± 22.9 (90 mins)	47.3 ± 20.4 (40 mins)	31.3 ± 10.2 (90 mins)	51.8 ± 13.6 (30 mins)	14.4 ± 5.7 (90 mins)	7.0 ± 0.2 † (30 mins)
 4NP BIS(2-THIENYL)PHOSPHINATE	69.2 ± 9.1 (90 mins)	67.5 ± 13.5 (10 mins)	17.2 ± 9.7 † (90 mins)	6.1 ± 0.4 † (50 mins)	9.3 ± 2.0 † (90 mins)	7.1 ± 3.2 † (50 mins)

† $p < .05$

Values are expressed as mean ± std. dev., n = 3 or 4

The following papers were published or prepared with the full or partial support of the Army Research Office during this reporting period:

1. Marquis, J.K.: Interactions of neuroactive drugs and group-specific ligands with purified human serum cholinesterase. Neurotoxicol. 6: 261-270 (1985).

2. Marquis, J.K. & E.E. Black: Activation and inactivation of bovine caudate acetylcholinesterase by trivalent cations. Biochem. Pharmacol. 34: 533-538 (1985).

3. Marquis, J.K.: Noncholinergic mechanisms of insecticide toxicity. Trends in Pharmacol. Sci. 6: 59-60 (1985).

4. Marquis, J.K.: Osmotically-induced trapping of terbium ions to monitor interior calcium binding sites in nerve membrane vesicles. Comp. Biochem. Physiol. 80C: 203-205 (1985).

5. Volpe, L.S., T.M. Biagioni & J.K. Marquis: In vitro modulation of bovine caudate muscarinic receptor number by organophosphates and carbamates. Tox. & Appl. Pharmacol. 78: 226-234 (1985).

6. Marquis, J.K. & E.B. Fishman: Presynaptic acetylcholinesterase. Trends in Pharmacol. Sci. 6: 387-388 (1985).

7. Fishman, E.B., G.C. Siek, R.D. MacCallum, L. Volicer & J.K. Marquis: Distribution of the molecular forms of acetylcholinesterase in human brain: alterations in dementia of the Alzheimer type. Annals of Neurol. 19: 246-252 (1986).

8. Marquis, J.K.: Contemporary Issues in Pesticide Toxicology and Pharmacology. S. Karger, Basel, Switzerland, 1986.

9. Marquis, J.K.: Chronic aluminum toxicity: Effects of aluminum on neurotransmitters. Neurobiology of Aging, 7: 547-548 (1986).

10. Marquis, J.K. & G.C. Siek: Sensitive populations and risk assessment in environmental health policy-making. In: Hazard Assessment of Chemicals - Current Developments, Ed. by J. Saxena, Vol. 6 (1988).

11. Marquis, J.K. & T.M. Biagioni: Selective inhibition of acetylcholinesterase and butyrylcholinesterase in human plasma, erythrocytes and cerebrospinal fluid. J. Pharm. Pharmacol., submitted 1988.

12. Marquis, J.K.: Alterations of central neurotransmitter function by aluminum. In: Trace Metals, Aging and Alzheimer Disease, NIA, In Press (1988).

13. Marquis, J.K.: Inhibition of bovine brain acetylcholinesterase by tetrahydroaminoaridine (Tacrine). Biochem. Pharmacol., In Press (1988).

14. Marquis, J.K. & R.D. MacCallum: Kinetics of inhibition of bovine brain acetylcholinesterase by organophosphate esters: spontaneous reactivation and oxime-mediated reactivation of chemically modified enzyme. J. Biochem. Toxicol., submitted 1988.

15. Marquis, J.K. (Ed.): A Guide to Applied Toxicology, S. Karger AG, Basel, Switzerland, In Press (1988).

16. Marquis, J.K. (Ed.): Cholinesterase Inhibition as an Indication of Adverse Toxicologic Effect. Risk Assessment Forum, U.S. EPA, In Press, 1988.

APPENDIX B

The following are published abstracts of papers presented at scientific meetings and describe work funded at least in part by the Army Research Office during this reporting period:

1. Marquis, J.K. & Y. Tsuzuki: In vitro activation of tyrosine hydroxylase by organophosphates. The Toxicologist 5: 142 (1985).

2. Fishman, E.B., G.C. Siek, T.M. Biagioni, R.D. MacCallum & J.K. Marquis: Distribution of the molecular forms of acetylcholinesterase in human brain. The Toxicologist 5: 142 (1985).

3. Marquis, J.K., G.C. Siek & T.M. Biagioni: Enzyme purification. Triservice Workshop on Enzymatic Decontamination, Ft. Detrick, MD, March, 1985.

4. Gallo, B.J., D.A. Gowenlock, J.W. Walker, J.K. Marquis & R.D. MacCallum: The production of prokaryotic cholinesterase. Triservice Workshop on Enzymatic Decontamination, Dt. Detrick, MD, March, 1985.

5. Mesulam, M-M., L. Volicer, J.K. Marquis, E.J. Mufson & R.C. Green: Regional variations in the cholinergic innervation of the primate cortical surface. Soc. of Neuroscience Abs., 11: 1237 (1985).

6. Mesulam, M-M., L. Volicer, J.K. Marquis, E.J. Mufson & R.C. Green: Cortical cholinergic innervation. Annals of Neurol. 18: 118a (1985).

7. Marquis, J.K. & E.B. Fishman: Presynaptic acetylcholinesterase: implications for understanding insecticide toxicity. J. Am. Coll. Toxicol. 4: 131 abs. (1985).

8. Marquis, J.K. & C.C. Cyr: Chemical modification of cholinesterase carbamylation kinetics by peripheral site ligands. The Toxicologist 6: 24 (1986).

9. Marquis, J.K. & R.D. MacCallum: Kinetics of inhibition and reactivation of mammalian brain acetylcholinesterase by organophsophinate esters. The Toxicologist, 7: 48 abs. (1987).

10. Marquis, J.K.: Chemical modification of peripheral site reactivity on mammalian brain acetylcholinesterase. 3rd ARO Neuroscience Workshop, Cashiers, NC, April, 1987.

11. Marquis, J.K. & R.D. MacCallum: Kinetics of inhibition and reactivation of mammalian brain acetylcholinesterase by organophosphate esters. Presented at 3rd ARO Neuroscience Program Review, Cashiers, NC, 1987.

12. Hanke, D.W., M.A. Overton, D. Wolfe, J.K. Marquis, C.N. Lieske & C. Burdick: *In vitro* induced reactivation and phosphorylation sites relative to *in vivo* oxime efficacy in organosphosphorus poisoning. Proceedings of American Chemical Society, Annual Meeting, New Orleans, LA, 1987.

13. Marquis, J.K.: Neurotoxicology of aluminum. Proceedings of American Chemical Society, Annual Meeting, New Orleans, LA, 1987.

14. Marquis, J.K. & G.C. Siek: Sensitive populations and risk assessment in environmental policy-making. J. Am. Coll. Toxicol., 6: 559 (1987).

15. Marquis, J.K.: Kinetics of inhibition of mammalian brain acetylcholinesterase by tetrahydroaminoacridine. Annual Meeting, Massachusetts Alzheimer's Disease Research Center, Boston, MA, 1987.

The following seminars and lectures were presented during this reporting period and describe work supported at least in part by the Army Research Office:

1. Triservice Workshop on Enzymatic Decontamination, Ft. Detrick, MD, March 26, 1985.
   Title: Enzyme purification.

2. Massachusetts Audubon Society, Norfolk, MA, October 26, 1985.
   Title: Current Issues in Pesticide Toxicology.

3. University of Massachusetts, Entomology Department, Amherst, MA, October 28, 1985.
   Title: Presynaptic AChE: Implications for Understanding Insecticide Toxicity.

4. Boston University School of Medicine, Technology Transfer Program, November 1, 1985.
   Title: Environmental Toxicology.

5. Northeast Chapter, SOT, Storrs, CT, October 12, 1985.
   Title: Presynaptic AChE: Implications for Understanding Insecticide Toxicity.

6. New England Environmental Conference, Tufts University, Medford, MA, March 22, 1986.
   Title: Approaches to Pesticide Use.

7. Northeastern University, Toxicology Program, Boston, MA, April 10, 1986.
   Title: Inhibition of acetylcholinesterase by organophosphinates.

8. National Institute on Aging, Bethesda, MD, September 24, 1986.
   Symposium on Trace Metals, Aging and Alzheimer's Disease.
   Title: Alterations of contral neurotransmitter function by aluminum.

9. Technology Transfer Program, Boston University School of Medicine, May 16, 1986.
   Title: Environmental Toxicology.

10. Boston University School of Medicine, Department of Pathology, November 14, 1986.
    Title: Cholinesterase molecular forms in human brain.

11. New England Interstate Water Pollution Control Commission, November 21, 1986.
    Title: Toxicity of Aldicarb (Temik) and Dinoseb: Health problems associated with acute and chronic exposure.

12. Golf Course Superintendents Association, Bass River, MA, April 7, 1987.
    Title: Environmental toxicology: issues of chronic toxicity.

13. U.S. Army Natick Research Laboratory, Natick, MA, April 22, 1987.
    Title: Chemical modification of mammalian brain acetylcholinesterase.

14. 57th Massachusetts Turfgrass Conference, Springfield, MA, March 1, 1988.
    Title: Environmental Toxicology - Issues of Chronic Toxicity.